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Copy Number Variation in Tourette Syndrome

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In the current issue of *Neuron*, Huang et al. (2017) provide new insights from a consortium study of Tourette syndrome pinpointing copy number variations that are involved in the genomic architecture and implicate genes of interest.

Tourette syndrome (TS) is a neuropsychiatric disorder primarily expressed as motor and/or phonic tics during childhood that has high heritability (Robertson et al., 2017). With an emphasis initially on family studies, and despite much effort using a series of study designs since the 1990s, the yield of genetic research has been modest (Willsey et al., 2017). In this context, the main results of a U.S.-led consortium effort to elucidate the genetics of TS with respect to copy number variations (CNVs) published in this current issue of *Neuron* (Huang et al., 2017) are notable. The main discoveries of this largest TS CNV study to date (2,434 unrelated cases, all of European ancestry) involve global enrichment of rare CNVs in TS, and the identification of two loci that achieved genome-wide significance: loss CNVs overlapping the *NRXN1* gene (i.e., hemizygous deletions presumably acting via haploinsufficiency) and gain CNVs overlapping the *CNTN6* gene (extra copies disrupting the *CNTN6* locus and sometimes also the adjacent *CNTN4* gene). While individually rare, collectively the CNVs overlapping these two loci comprised 24 of the TS cases, or nearly 1% of the sample investigated. Because so few comparable CNVs were found overlapping these genes in the 4,093 control samples used, the odds ratios (OR) for each are impressive: 20.3 (95% CI 2.6–156.2) for exonic *NRXN1* deletions and 10.1 (95% CI 2.3–45.4) for exonic duplications involving *CNTN6*.

The findings confirm and extend those for *NRXN1* deletions reported in earlier smaller studies of TS (Robertson et al., 2017), and provide novel insights for TS with respect to the contactin gene findings. Both genes are known to be implicated in CNV studies of other major neuropsychiatric disorders (Lowther et al., 2017; Oguro-Ando et al., 2017). Thus, the unifying theme is one of variable neuropsychiatric expression. Understanding this variability is one of the main challenges in genetics research and will be essential for determining the pathways from genetic changes to clinically recognizable diseases.

What do the results from this consortium study tell us about the genetics of variable expression and about the pathogenesis of TS? It is likely that the expressed phenotype may be affected by the extent to which the CNV overlaps the gene of interest, i.e., the site of the mutation (Uddin et al., 2014), and by other genetic variants elsewhere in the genome. For this study, by combing through the supplemental material (Huang et al., 2017), it is possible to examine additional rare CNVs, previously shown to play a potential role in expression of *NRXN1* deletions (Lowther et al., 2017), and available phenotype information. These data show different patterns for the 12 subjects with CNVs overlapping *NRXN1* and the 12 with CNVs overlapping *CNTN6*. As is typical for genetic studies and in neuroscience, the rare can inform the many.

With respect to the site of the *NRXN1* mutations in TS subjects (8 male, 4 female), the deletions ranged in size from 163 to 825 kb. All involved the proximal (5′) region and the first 8 of the 22 exons of this large gene, and all would be considered clinically pathogenic using standard criteria for adjudicating CNVs (Lowther et al., 2017). Eleven of these 12 *NRXN1* deletions are confined to the first four exons and also appear to overlap the AK127244 long non-coding RNA (lncRNA) immediately centromeric (5′) to the NRXN1-alpha transcript (not shown in Figure 4 in Huang et al., 2017). Importantly, this is similar to the pattern observed for pathogenic *NRXN1* deletions reported by clinical labs where most of the patients were referred with developmental disability, and for the majority of *NRXN1* deletions where enrichment for these deletions has been reported in studies of schizophrenia (Lowther et al., 2017). In addition to this "unseen" brain-expressed non-coding gene of unknown function, there were additional rare genic (5 loss, 9 gain) CNVs recorded for 8 of the 12 TS subjects with *NRXN1* deletions (Table S4 in Huang et al., 2017), four of which were >100 kb, including a 611 kb loss CNV at 3p25.3 overlapping five genes including CAV3. Although annotations were not provided in Table S4, this CNV would likely be deemed clinically pathogenic (Costain et al., 2013; Uddin et al., 2014). For the 11 subjects with clinical data available, all but one had additional conditions recorded, five of which were in the form of notes documenting neurodevelopmental conditions including developmental delays (Table S5 in Huang et al., 2017). This is comparable to the

phenotypic complexity reported for clinically pathogenic *NRXN1* deletions; nearly half of the 44 described had conditions such as autism spectrum disorders (ASD), attention deficit hyperactivity disorder (ADHD), obsessive compulsive disorder (OCD), neuromuscular problems, speech problems, and interestingly two with TS, in addition to developmental delay/intellectual disability (Lowther et al., 2017). The prevalence of exonic *NRXN1* deletions identified through standard clinical microarray testing of 19,263 patients, while significantly greater (0.21%) than in 15,264 controls (OR = 8.14 [95% CI 2.91–22.72]) (Lowther et al., 2017), was less than that found in the TS study (Huang et al., 2017).

Although the TS diagnostic endpoint was the same as for *NRXN1*, the genetic and clinical picture was different for the *CNTN6* finding, reflecting the lower penetrance expected for duplications and the potential importance of the regional genomic architecture with respect to contactin genes at this chromosome 3p26.3 locus. Of the 12 subjects (11 male) with gain CNVs overlapping *CNTN6*, three with similar breakpoints extended to also overlap the nearby *CNTN4* gene (Figure 4 and Tables S4 and S5 in Huang et al., 2017) and a fourth subject had a second 301 kb gain CNV overlapping *CNTN4* not shown in Figure 4 (Huang et al., 2017). Also not displayed were four other (male) TS subjects with 227 to 928 kb gain CNVs overlapping *CNTN4* but not *CNTN6*. These help to place in a CNV (versus a gene-only) context the statistical association for *CNTN4* presented (Table S6 in Huang et al., 2017). In addition to these regional findings, 8 of the 12 subjects with *CNTN6* gains had other genic CNVs (10 gains, 2 losses; 7 > 100 kb in length, Table S4 in Huang et al., 2017). None of the 12 had neurodevelopmental conditions noted, and four had neither OCD nor ADHD recorded (Tables S4 and S5 in Huang et al., 2017). Notably, the lone female TS subject with a duplication overlapping *CNTN6* had three other genic CNVs recorded, including a recurrent 395 kb (C to D) distal 22q11.2 deletion that would clinically be considered as likely pathogenic. Previous studies emphasize the importance of considering deletions and duplications involving *CNTN6* separately, the variable expression and reduced penetrance of these CNVs, and the related expectations that many would be inherited and found in control samples (Hu et al., 2015; Oguro-Ando et al., 2017; Mercati et al., 2017).

One could also draw attention to the potential effects of sex on phenotype. The three controls with either *NRXN1* deletions (n = 1) or *CNTN6* duplications (n = 2) were all female with length of CNVs on the small side, though >100 kb (Huang et al., 2017). This is in line with the general finding that the sex differences being noted for rare CNVs in neurodevelopmental disorders involve a tolerance in females to the effects of CNVs. Expression in females may require additional genetic loading that may come in the form of longer and/or additional rare genic CNVs or other variants. Such data are rarely provided for controls.

In addition to ubiquitous issues related to control set collection (Bassett et al., 2010), CNV interpretation requires consideration of global issues relevant to the substantial efforts that go into consortia to study the genetics of common complex conditions like TS. The main significant results are largely confined to rare CNV findings. Further details about those deemed "pathogenic" would be of clinical relevance (Costain et al., 2013), e.g., recurrent 15q11q13 duplications found in 2 TS subjects and no controls (Table S7 in Huang et al., 2017). As for most consortia, there are very limited phenotypic details available (Bassett et al., 2010). Particularly disappointing here and rather surprising for a pediatric condition like TS, there are no consistent data on global developmental level, and data on age, growth, or medical conditions were not provided. Data were missing for the two comorbid conditions recorded (OCD, ADHD) for nearly one in four subjects. There are also no data on the *de novo* or inherited status of interesting CNVs, or on family history, including whether ascertainment involved probands from apparent singleton or multiplex families (Bassett et al., 2010). Also, such consortium studies usually involve an expedient design focused on proband DNA sample collection that precludes the ability to return to the subjects for further phenotyping and family work-up that would help interpret the results (Bassett et al., 2010). Other studies indicate that these rare CNVs are often inherited, with a broad spectrum of associated expression, and complex families with relevant conditions on both sides (Hu et al., 2015; Lowther et al., 2017). Calling CNVs using algorithms remains a complex task—these are not direct determinations of CN loss or gain. For example, some closely spaced CNVs may require "knitting" together, and others, e.g., a large 17q25.1 gain (Table S6 in Huang et al., 2017), appear common (involving >1% of the sample). Due to the rarity of individual CNVs of interest, the numerators are small and denominators large, with a need for large control sets in order to be able to obtain "genome-wide significance." Whether subjects were previously reported elsewhere is unknown, of potential import when small numbers are involved (Bassett et al., 2010). Ascertainment biases differ from site to site, and thus "batch effects" may not be confined to the lab. As for most such consortia, sample collection and analyses are focused on individuals of European descent.

Notwithstanding these caveats, studies such as this are important to help inform potential clinical outcomes, and the results will be considered in clinical CNV interpretation and for genetic counseling (Costain et al., 2013). Current American College of Medical Geneticists (ACMG) recommendations for clinical microarray testing are confined to individuals at any age with developmental disability, autism and/or multiple congenital anomalies (Miller et al., 2010). The significant burden reported for ACMG pathogenic CNVs in this TS study (Huang et al., 2017) suggests that there may be clinical genetic testing implications for some patients. Going forward, reconsidering consortium study design to include detailed phenotype and basic family genetic context will be beneficial on a clinical and on a research basis. The significant findings of this TS study are rather unusual for neuropsychiatric conditions in that they involve individual CNVs that predominantly overlap one large, complex gene, compared with the multigenic recurrent CNVs usually implicated. Nonetheless, as noted above, CNVs can in a single hit knock out multiple isoforms and regulatory regions (Uddin et al., 2014), and additional variants are likely to be involved (Lowther et al., 2017; Mercati et al., 2017). While there may be a unifying

pathogenesis involving a neurexin *trans*-synaptic system that could include *CNTNAP2* (Clarke and Eapen, 2014), this is not necessarily the case (Willsey et al., 2017). Further consideration of the global genomic architecture will be essential for understanding the likely multiple pathways and interactions involved in the TS phenotype. This will include whole-genome sequencing, with integration of results for rare variants affecting genomic structure (e.g., additional CNVs) and DNA sequence in coding and non-coding regions, and common genetic (e.g., polygenic) background. The results here for TS however are in line with the typical findings for neuropsychiatric conditions, including neurodevelopmental disorders such as intellectual disability, ASD, schizophrenia, speech disorders, ADHD, epilepsy, etc. Genetic heterogeneity within and between patients, and clinical heterogeneity with reduced penetrance of individually important rare variants, are the norm.

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Live or Die? Depends on Who You Are

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In this issue of *Neuron*, Welsbie et al. (2017) and Norsworthy et al. (2017) implicate the transcription factor Sox11 as a key player after optic nerve injury—in DLK signaling of RGC cell death, and in RGC regeneration and survival but only in certain RGCs.

During development, neurons are born, their cell fate specified, and they differentiate, extending axons to target regions to form connections on target cells. After differentiation, through naturally occurring cell death, the number of neurons in a given population is adjusted, thought to reduce competition for synaptic space during the establishment of neural circuits. Following axon injury to the adult mammalian central nervous system (CNS), axons retract, unwanted cell death ensues, and without efforts for repair, circuits are ultimately disconnected. In the last few years, a number of studies have pinpointed both intrinsic and extrinsic molecules and their signaling pathways that can implement both axon regeneration and neuronal survival in injured neurons (Crair and Mason, 2016; He and Jin, 2016). However, the molecular mechanisms that mediate survival versus death of specific types of neurons are still unclear. Further, if injured adult neurons do survive, one strategy for repair is to manipulate molecules present in the adult but suppressed, such as mTOR, inhibited by PTEN, that then stimulates axon extension. An alternative strategy is to reprogram or reactivate factors that function in developmental growth programs. In this issue of *Neuron*, two papers report on each of these strategies, one elucidating pathways that lead to retinal ganglion cell (RGC) death after injury and the other demonstrating developmental programs that can be harnessed for axon regeneration but only for certain types of RGCs.